#17
B. Webb
12/15/01

BEFORE THE BOARD OF APPEALS AND INTERFERENCES
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Arnold et al.	Group Art Unit: 1655
Serial No. 09/586,156	Examiner: Lu, F.
Filed: June 2, 2000	Attorney Docket No. IN-0016-1
For: *Duplex Probes for Hybridization Reactions*	

CERTIFICATE OF MAILING
I hereby certify that this corr is being deposited with the US Postal Service as First Class Mail in an envelope addressed to the Comm of Patents, Washington, D.C. 20231 on November 12, 2001

Signed _____
Richard Osman

REPLY BRIEF ON APPEAL

The Honorable Board of Appeals and Interferences
United States Patent and Trademark Office
Washington, D.C. 20231

Dear Honorable Board:

The Examiner's Answer dated 10/3/01 is expressly and entirely premised on the Examiner misunderstanding what is meant by the technical term "complementary". Remove this technical misunderstanding, and all of the Examiner's rejections fall.

I. CLAIMS 1, 2, 8, 11, 15, 16, 22 and 23 ARE PATENTABLE UNDER 35USC102(b).

Complementary polynucleotide strands can hybridize by "Watson-Crick" base pairing (A-T and C-G pairing). For example, the sequence "5'-A-G-T-3'" can hybridize with its complementary sequence, "3'-T-C-A-5" (Specification, p.5, lines 3-4). Note that complementary sequences are actually antiparallel (reverse complements): to hybridize by Watson-Crick base-pairing, the complementary sequence necessarily has the complementary nucleotides (A for T, C for G, etc.) in

reverse 5' - 3' orientation. Hybridization of complementary sequences by Watson-Crick base pairing is what generates the double-helix structure of DNA.

While this may seem elementary, it is the basis of our Appeal. In the unlikely event the Board is not familiar with this conventional, though technical terminology, we append page 21 from the now classic textbook by Watson himself (*Recombinant DNA, Second Edition*, 1983, Scientific American Books) showing what complementary base pairing looks like. Note that each T base of the left strand is necessarily paired with an A base of the right strand, each C base of the left strand is paired with a G base of the complementary right strand, and so on. Note also that the complementary strands are necessarily antiparallel - the one on the left is 5'-3' (top to bottom), whereas the complement on the right necessarily is 3'-5' (top to bottom).

Also enclosed is a printout of Chapter 4 of the current, online version of the textbook, *Molecular Cell Biology* (WH Freeeman & Co.), coauthored by another Nobel Laureate, which also explains how Watson-Crick base pairing works and what is meant by "complementary" sequences (see, first and second paragraphs on p.3 of 7). "Complementary" is a term of art, referring to antiparallel sequences which hybridize by Watson-Crick base pairing to form the classic double helix structure: "In natural DNA, A almost always hydrogen bonds with T and G with C, forming A·T and G·C base pairs often called *Watson-Crick* base pairs. Two polynucleotide strands, or regions thereof, in which all the nucleotides form such base pairs are said to be complementary." (supra, p.3, lines 10-13).

In the fourth and fifth paragraphs of the same page, the authors describe other possible DNA structures that might exist in cells, including the relatively arcane A-, and Z-forms and triple-stranded DNA structures formed by "Hoogsteen" binding. Triplex formation by Hoogsteen binding can occur only under very particular conditions, wherein a third polynucleotide strand binds to the major groove of a Watson-Crick double helix. In this tenuous interaction, the bases of the third strand form what are called "Hoogsteen base pairs" with the back sides of purine bases of the double helix (see, Fig.2 on p.40 of Molecular Biology and Biotechnology, Ed. Robert A Meyers, 1995, enclosed). Note that one strand of the target double helix must have a long stretch of purine bases for this to work. Note also

that Hoogsteen binding does not occur between complementary strands, as the term is used in the art and our Specification, to either strand of the double helix. In fact, Hoogsteen binding even occurs between purine (A or G) bases of the third strand and purine bases of the double helix (see, Frank-Kamenetskii, et al., Ann Rev Biochem 1995;64-95, abstract enclosed) - something that is the antithesis of complementarity, which requires a purine (A or G) pairing with a pyrimidine (T or C, respectively).

On p.5 of his Action, the Examiner proposes some contradiction between our explanation of the cited art describing Hoogsteen binding and our invention, which requires hybridization of complementary sequences. There is no contradiction, though there is plenty of irony in the Action's false and condescending statement at the bottom of p.5. That the Examiner misapprehends the difference between Hoosteen binding and Watson-Crick binding of complementary sequences is demonstrated again on the next page, where the Examiner writes, "at least two types of hydrogen pairing exist in nucleic acid double helix, Watson-Crick type of hydrogen pairing and non-Watson-Crick interaction known as Hoogsteen pairing" (Answer, p.6, lines 3-5). This is nonsense. Double helices are formed and maintained exclusively by Watson-Crick base pairing. Hoogsteen pairing is limited to a particular kind of triplex (supra) and never occurs in a double helix.

Furthermore, the Examiner's attempt to redefine "complementary" to encompass non-complementary sequences (Answer, p.6, lines 6-13 of his Answer), is repugnant to its conventional usage in the art (supra), and our Specification nowhere suggests such a deviation from conventional terminology. The cited paragraph says that complementary refers to "natural binding of polynucleotides under permissive salt and temperature conditions by base pairing. For example, "A-G-T" binds to the complementary sequence "T-C-A." This can only mean what is conventionally meant by complementary (supra). Hoogsteen binding has never been shown to be "natural" and the exemplary AGT-TCA binding can never be Hoogsteen, which requires a target purine sequence (here, the target T and C bases are both pyrimidines). Furthermore, the usage of the term complementary throughout the Specification including Figures 3-7 and all the Examples is consistent with its conventional usage and applicable only to Watson-Crick base pairing of antiparallel sequences. Fig.3, for example, shows complementary probes targeting a hairpin duplex, wherein each probe is the reverse complement of a

strand of the duplex portion of the hairpin, and hybridizes with the strand by Watson-Crick base-pairing (e.g. p.15, lines 14-18).

The Examiner's "third" point (Action, p.6, lines 14-16) is both incorrect and nonsensical. Our interpretation is correct: the orientation of the polyT probe binding is parallel (not complementary) with the polyA of the duplex (see Fig.2) in Bates' A30-A30·T30 triplex, and the polyA probe binding in the A30-A30·T30 triplex (p.3630) is antiparallel with the polyA - not with the polyT. Bates never teaches the "A30-T30·A30" proposed by the Examiner, and for good reason: it is an impossible construct – the third "A30" strand can only bind a purine (e.g. "A30") backbone - never a pyrimidine (e.g. "T30") sequence as proposed.

The Examiner's response to our footnoted remarks is also factually incorrect and nonsensical. On p.6, line 17 - p.7, line 7, the Examiner persists with his "having the potential to be complementary" phraseology. We maintain that "potential to be complementary" is an oxymoron - a polynucleotide either is complementary to another or it is not. Furthermore, on p.7, lines 8-18, the Examiner again mischaracterizes Bates's molecules. The Examiner claims that Bates does describe an HD1-HD2·HD3 system and alleges that there is no difference between an HD3-HD2·HD1 and a HD1-HD2·HD3 system. The Examiner is incorrect on both counts.

HD1, HD2 and HD3 are defined in Bates's Figure 2. Note that HD1 and HD2 are complementary and antiparallel. They can only hybridize by Watson-Crick base pairing to form a double helix. HD3 has the same sequence as HD2, but in opposite orientation. HD3 can hybridize by Watson Crick base pairing with neither HD1 nor HD2. HD3 can only interact with the purine stretch of the HD1 strand of an HD1·HD2 double helix. In Bates' nomenclature, X-Y·Z means that Y·Z is a conventional double helix wherein X binds the Y strand by Hoogsteen binding (Bates, p.3628, col.1, lines 15-17). Hence, by Bates' nomenclature, the only Hoogsteen triplex that can be formed with these oligos is a HD3-HD1·HD2 triplex[1].

Consider, then, the Examiner's contentions. First, his proposed HD3-HD2·HD1 (HD3

[1] The nomenclature requires fastidiousness: even our own prior footnote 2 contained an error, reversing the HD2 and HD1 strands of HD3-HD1·HD2.

Hoogsteen binding an HD2·HD1 double helix) is *not* the same as HD1-HD2·HD3 (HD1 Hoogsteen binding an HD2·HD3 double helix). Second, Bates does not and can not describe the alleged HD1-HD2·HD3 because it is not a physically possible structure. Here, (referring to Bates' nomenclature) HD2 would form a double helix with HD3 and then HD1 would Hoogsteen bind with the HD2 strand of the double helix. Both bindings are impossible: HD2 and HD3 can not form a double helix because they are not complementary and HD1 can not Hoogsteen bind to HD2 because HD2 does not have a stretch of purine bases.

The Examiner's contentions are unsound. The structural requirements of our claims are neither met nor suggested by the cited Bates et al. (Nucleic Acids Res. 23, 3627-3632, 1995), which describes the entirely unrelated Hoogsteen triplex formation (p.3628, col.1, line 16). The three triplex-forming systems of Bates are shown in her Table 1: the cited reactions are (1)Bt-T30/A30/T30, (2)Bt-AY/AU/Pso-20 and (3)Bt-HD1/HD2/HD3. Figure 2 describes each of these molecules: in each case, you have the third single stranded molecule binding the backside of the purines of the immobilized double stranded molecule. Note that the third strands are always pyrimidine polymers (T30, Pso-20 or HD3; see Fig.2). There is no complementarity, as expressly required by our claims. Note that even with Bates' polyT/polyA homopolymers, the orientation of the polyT probe binding is parallel (not complementary) with the polyA of the duplex (see Fig.2) and the polyA probe binding of the A30-A30-T30 triplex (p.3630) is antiparallel with the polyA - not with the polyT. Bates' solid phase Hoogsteen binding assay provides a useful system for studying the kinetics of this phenomenon, however it is inapplicable to other than pyrimidine probes and purine rich targets, and it is not a hybridization assay as claimed. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

II. CLAIMS 1, 2, 8-13 and 15-23 ARE PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence

flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

Note that our claims recite only a single probe. Where the target is single-stranded, the probe is double-stranded. Then, all subsequent references to the probe refer to *the* antecedent double-stranded probe. Our claims do not encompass a single-stranded target hybridizing with a single-stranded probe that happens to have complementary ends and can otherwise form a stem-loop structure. We maintain that our claims absolutely require complementary triplex formation: a double-stranded molecule (target or probe) must hybridize with a single-stranded molecule (the other of the target or probe) to form a triplex. The Examiner's contrary proposal (Answer, p.12, lines 15-16) is not reconcilable with the recitals of our claims. Redrafting our claims to support the Examiner's proposed construction would require recital of something like "a first double-stranded probe which upon hybridization interconverts to a second, single stranded probe." Our claims require that if the probe is double-stranded before it hybridizes, it must be *the* same double-stranded probe after it hybridizes.

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Combining

the two references would provide no more than the use of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claims 15-23 are further removed from the cited art as these claims expressly require that the probe which hybridizes to the immobilized target be double-stranded. The cited art suggests nothing but single-stranded molecules hybridizing to single-stranded target. Though Tyagi's probes can exist in a partially-double stranded form, they are not double-stranded when hybridized to target - in fact, the target hybridizing portion of Tyagi's probes do not have a complementary sequence within the probe.

III. CLAIM 3 IS PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Anderson et al (1985 Nucleic Acid Hybridization: a practical approach p. 86-109). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the

probe).

Note that our claims recite only a single probe. Where the target is single-stranded, the probe is double-stranded. Then, all subsequent references to the probe refer to *the* antecedent double-stranded probe. Our claims do not encompass a single-stranded target hybridizing with a single-stranded probe that happens to have complementary ends and can otherwise form a stem-loop structure. We maintain that our claims absolutely require complementary triplex formation: a double-stranded molecule (target or probe) must hybridize with a single-stranded molecule (the other of the target or probe) to form a triplex. The Examiner's contrary proposal (Answer, p.12, lines 15-16) is not reconcilable with the recitals of our claims. Redrafting our claims to support the Examiner's proposed construction would require recital of something like "a first double-stranded probe which upon hybridization interconverts to a second, single stranded probe." Our claims require that if the probe is double-stranded before it hybridizes, it must be *the* same double-stranded probe after it hybridizes.

The Anderson reference is cited for no more than the reuse of filters and probes after hybridization. Combining the two references would provide no more than the reuse of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

IV. CLAIMS 14 and 24 ARE PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996), Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994) and Brown et al. (US Patent No. 5,807522). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching

moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

Note that our claims recite only a single probe. Where the target is single-stranded, the probe is double-stranded. Then, all subsequent references to the probe refer to *the* antecedent double-stranded probe. Our claims do not encompass a single-stranded target hybridizing with a single-stranded probe that happens to have complementary ends and can otherwise form a stem-loop structure. We maintain that our claims absolutely require complementary triplex formation: a double-stranded molecule (target or probe) must hybridize with a single-stranded molecule (the other of the target or probe) to form a triplex. The Examiner's contrary proposal (Answer, p.12, lines 15-16) is not reconcilable with the recitals of our claims. Redrafting our claims to support the Examiner's proposed construction would require recital of something like "a first double-stranded probe which upon hybridization interconverts to a second, single stranded probe." Our claims require that if the probe is double-stranded before it hybridizes, it must be *the* same double-stranded probe after it hybridizes.

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Brown is cited for the use of poly-L-lysine to coat microarray substrates. Combining the three references would provide no more than the use of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction on a substrate coated with poly-L-lysine. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claim 24 is further removed from the cited art as this claim expressly requires that the probe which hybridizes to the immobilized target be double-stranded. The cited art suggests nothing but single-stranded molecules hybridizing to single-stranded target. Though Tyagi's probes can exist in a partially-double stranded form, they are not double-stranded when hybridized to target - in fact, the target hybridizing portion of Tyagi's probes do not have a complementary sequence within the probe.

Appellants respectfully request reversal of the pending Final Action by the Board of Appeals.

Appellants petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any necessary fees or credit any overpayments associated with this communication to our Deposit Account No. 19-0750 (order no. IN-0016-1).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

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encl. Molecular Biology and Biotechnology, Ed. Robert A Meyers, 1995, p.39-40 (2 p.)
Molecular Cell Biology, Lodish et al., Online Edition, Chap.4 (7 p.)
Recombinant DNA, Second Edition, Watson et al., 1983, p.21 (1 p.)
Frank-Mamenetskii, et al., Ann Rev Biochem 1995;64-95, abstract (1 p.)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Arnold et al.

Serial No. 09/586,156

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PLEASE DELIVER TO SPE GARY JONES

Examiner: Lu, F.

Group Art Unit: 1655

Attorney Docket No. IN-0016-1

CERTIFICATE OF TRANSMISSION
I hereby certify that this corr is being transmitted by facsimile to the Comm for Patents at (703) 872-9303 on December 10, 2001.

Signature _____
Richard Aron Osman

TRANSMITTAL

The Commissioner for Patents
Washington, DC 20231

Dear Commissioner:

Transmitted herewith is a copy of our reply brief originally mailed to the Commissioner of Patents, Washington, D.C. 20231 on November 12, 2001 as shown on the certificate of mailing. Accordingly, no extensions of time or fees are necessary.

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

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